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Substitute Specification

**HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL
CROSSLINKING REAGENTS****RELATED APPLICATIONS**

This application is a divisional of U.S. patent 6,800,728, patent application No.: 09/815,978, filed March 22, 2001 entitled "HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS" which claims priority to U.S. provisional patent application No. 60/191,186, filed March 22, 2000, to Schwartz, entitled "NOVEL HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS." The disclosures of the above-referenced applications are incorporated herein in their entirety.

FIELD OF THE INVENTION

The present disclosure may be applied in general to the field of chemistry, more particularly in the area of crosslinking reagents.

BACKGROUND OF THE INVENTION

Methods to crosslink biomolecules such as proteins, oligonucleotides and carbohydrates to each other, to radioactive and non-radioactive metal chelates, to drugs and to surfaces have allowed development of both in vitro and in vivo diagnostic assays as well as in vivo therapies. A wide variety of methods have been developed and reviewed (Greg T. Hermanson, Bioconjugate Techniques, Academic Press).

There are a limited number of crosslinking couples, i.e., maleimide/thiol and bromoacetamide/thiol, that are routinely used to prepare conjugates for diagnostic and therapeutic uses. These reagents have limitations in that at high protein concentrations (i.e., >5 mg/mL) protein/protein crosslinking may occur. Also, the maleimido-modified moieties have a limited half-life due to hydrolysis at neutral and basic pH. Incorporation of thiol moieties on biomolecules requires both a coupling and a subsequent activation step. The resultant thiol-modified proteins can readily oxidize to form disulfide polymerized proteins. Also macromolecules containing disulfide bonds, i.e., antibodies, are readily